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ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH STORED SORGHUM (*Sorghum bicolor* L. Moench) SEEDS IN LAFIA, NASARAWA STATE, NIGERIA

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ABSTRACT
A study was carried out to isolate and identify fungi associated with stored sorghum seeds in Lafia, Nasarawa State, Nigeria. A total of 150 seeds each of brown and white sorghum randomly collected from 5 storage facilities, were surface sterilized and cultured on Potato Dextrose Agar using the direct plating method, for a period of 3 days. Pure cultures of fungi emerging from cultured sorghum seeds were identified using cultural and microscopic examination of growth and reproductive features. A total of 83 fungi consisting of 72 (86.75%) Ascomycota, 10 (12.05%) Zygomycota, and 1 (1.21%) Oomycota, were isolated from both white and brown sorghum varieties. Fungi belonging to the genus *Alternaria* were the most occurring with 53.01%, followed by *Mucor* with 12.05%, *Curvularia*, 8.43%, *Aureobasidium* and *Aspergillus* with 7.23% respectively, *Fusarium*, 4.81% and *Penicillium*, 2.40%. The least occurring fungi genera were *Pythium*, 1.21%, *Colletotrichum*, 1.21%, *Chrysosporium*, 1.21%, and *Chrysonilia*, 1.21%. White sorghum had the highest number of fungal isolates 60(40.0%) compared to brown sorghum 49(32.60%). Differences in frequency of fungal infections among the studied sorghum varieties were not significant ($P \leq 0.05$). The extensive occurrence of fungi on the stored sorghum suggests the availability of suitable relative humidity and temperature in storage facilities, favourable for growth and development of the observed fungi. There is need for emphasis on proper drying and the provision of adequate grain storage facilities in the study area.

***Keywords*:** *Fungi, Sorghum Varieties, Storage Facilities, Post-harvest, Lafia*

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) a member of the grass family *Poaceae* is quantitatively the world's fifth largest most important cereal after wheat, maize, rice and barley (Taylor, 2003). It is considered a staple for several homes in Africa and Asia (Ratnavathi and Patil, 2013). Nigeria is currently ranked the world's second largest producer of sorghum, with an annual output of 6.94 million tonnes (Proshare, 2018). Four million hectares of land in Nigeria are under Sorghum cultivation, making it the most cultivated and consumed cereal in the country (Singh *et al.*, 1997). Sorghum is used in many forms as an important starchy food for human and animal consumption, particularly in Northern Nigeria (Makun *et al.*, 2009).

Poor product quality associated with mould infestation of stored seeds is a major constraint to the cultivation and marketing of seed crops and their products in Nigeria and most parts of Africa. It has been estimated that Africa loses about sixty-seven ($67) million dollars annually from export rejects due to high levels of mycotoxins in food and agricultural produce (Ojochenemi *et al.*, 2016), owing largely to unavailability of adequate post-harvest storage facilities. Nigeria loses an estimated 2.4 billion tons of food yearly to poor harvest and storage facilities (Faajir, 2017).

Sorghum grains are ideal substrates for mould growth when poorly dried and stored (Martin *et al.*, 2006). The mycoflora of Sorghum seeds and seed products have been extensively studied in different parts of Nigeria (Kange *et al.*, 2015; Ojochenemi *et al.*, 2016; Hertveldt, 2016), however, there is currently no report on studies to evaluate fungal infection of stored sorghum seeds in Nasarawa State, Nigeria. This study is therefore aimed at bridging the knowledge gap regarding the occurrence and identification of fungi associated with post-harvest infection of stored sorghum seeds in Lafia, Nasarawa State, Nigeria. The findings of this study shall serve as a veritable tool in enhancing seed quality and improving the market value of stored sorghum seeds in the study area.

MATERIALS AND METHODS

Sample Collection

Thirty seeds each of brown and white Sorghum were randomly collected from 5 storage facilities in Lafia, Nasarawa State Nigeria, and conveyed in sterile polyethylene bags to the Botany Laboratory of Federal University Lafia, for further studies.

Isolation and Identification of Fungal Isolates from Sampled Sorghum Seeds

Seed samples were surface sterilized by dipping in 1% hypochlorite solution for one minute, followed by three

successive rinses in sterile distilled water. Isolation of fungi was done using the direct plating method described by Pardo *et al.* (2004). Ten surface-sterilized seeds belonging to each sorghum variety were separately plated on prepared media (Potato Dextrose Agar complemented with 0.5% chloramphenicol), and incubated at room temperature for three days to allow growth of any tissue-dwelling fungi. Pure cultures of isolated seed borne fungi were identified based on cultural and microscopic characteristics (Samson *et al.*, 2010).

Experimental Design

Experimental units were set up using the Completely Randomised Design (CRD) with 2 replicates.

Data Analysis

Data obtained from the study were subjected to Analysis of Variance (ANOVA) at 5% significance level.

RESULTS

A total of 44 fungi belonging to the Divisions Ascomycota and Zygomycota were isolated from seeds of the white sorghum variety (Table 1). Members of the Division Ascomycota were the most occurring, 40/44 (90.91%) compared to Zygomycota, 4/44 (9.09%).

Thirty nine fungal isolates belonging to the Divisions Ascomycota, Zygomycota and Oomycota were obtained from seeds of the brown sorghum variety (Table 2). Members of the Ascomycota were the most occurring, 32/39(82.05%), followed by Zygomycota, 6/39(15.39%) and lastly Oomycota, 1/39(2.56%).

Fungi belonging to the genus *Alternaria* were the most occurring with 53.01%. The least occurring fungal genera were *Pythium* (1.21%), *Colletotrichum* (1.21%), *Chrysosporium* (1.21%), and *Chrysonilia* (1.21%) (Table 3).

The evaluated sorghum varieties showed variations in percentage frequency of infection by storage fungi (Table 4, Plate 1). White sorghum had the highest number of 60(40.0%) fungal colonies per 150 seeds, compared to 49(32.60%) on brown sorghum. Differences in frequency of fungal infections among the studied sorghum varieties were not significant ($P \leq 0.05$) (Table 4).

Table 1: Fungi Associated with Stored Seeds of the White Sorghum Variety in Lafia

S/N	Isolate Code	Identity	Division
1.	WS1A2	*Curvularia sp.*	Ascomycota
2.	WS1B1	*Penicllium sp.*	Ascomycota
3.	WS1B2	*Fusarium sp.*	Ascomycota
4.	WS1B3	*Alternaria sp.*	Ascomycota
5.	WS1C2	*Alternaria sp.*	Ascomycota
6.	WS4A1	*Curvularia geniculata*	Ascomycota
7.	WS4A2	*Curvularia geniculata*	Ascomycota
8.	WS4A3	*Mucor sp.*	Zygomycota
9.	WS4A4	*Alternaria sp.*	Ascomycota
10.	WS4A5	*Alternaria sp.*	Ascomycota
11.	WS4A6	*Alternaria sp.*	Ascomycota
12.	WS4A7	*Mucor sp.*	Zygomycota
13.	WS4C1	*Alternaria sp.*	Ascomycota
14.	WS4C2	*Curvularia geniculata*	Ascomycota
15.	WS4C3	*Alternaria sp.*	Ascomycota
16.	WS4C4	*Fusarium sp.*	Ascomycota
17.	WS4C5	*Aspergillus niger*	Ascomycota
18.	WS4C6	*Fusarium equiseti*	Ascomycota
19.	WS11A1	*Alternaria sp.*	Ascomycota
20.	WS11A2	*Aureobasidium pullulans*	Ascomycota
21.	WS11A3	*Alternaria sp.*	Ascomycota
22.	WS11B1	*Alternaria sp.*	Ascomycota
23.	WS11B2	*Alternaria infectoria*	Ascomycota
24.	WS11B3	*Mucor sp.*	Zygomycota
25.	WS11C1	*Aspergillus niger*	Ascomycota
26.	WS11C3	*Alternaria sp.*	Ascomycota
27.	WS11C4	*Alternaria sp.*	Ascomycota
28.	WS11C7	*Alternaria sp.*	Ascomycota
29.	WS14A4	*Mucor sp.*	Zygomycota
30.	WS14C1	*Alternaria sp.*	Ascomycota
31.	WS14C2	*Curvularia geniculata*	Ascomycota
32.	WS14C3	*Alternaria sp.*	Ascomycota
33.	WS14C4	*Alternaria sp.*	Ascomycota
34.	WS14C5	*Alternaria sp.*	Ascomycota
35.	WS14C6	*Alternaria sp.*	Ascomycota
36.	WS14C7	*Alternaria sp.*	Ascomycota

37.	WS22A1	*Aspergillus sydowii*	Ascomycota
38.	WS22B1	*Alternaria sp.*	Ascomycota
39.	WS22B2	*Alternaria sp.*	Ascomycota
40.	WS22B3	*Alternaria sp.*	Ascomycota
41.	WS22B4	*Alternaria sp.*	Ascomycota
42.	WS22C1	*Alternaria sp.*	Ascomycota
43.	WS22C2	*Colletotrichum sp.*	Ascomycota
44.	WS22C3	*Alternaria sp.*	Ascomycota

Table 2: Fungi Associated with Stored Seeds of the Brown Sorghum Variety in Lafia

S/N	Isolate Code	Identity	Division
1	BS1A1	*Aureobasidium pullulans*	Ascomycota
2	BS1A3	*Aspergillus flavus*	Ascomycota
3	BS1A4	*Aspergillus niger*	Ascomycota
4	BS1A5	*Alternaria sp.*	Ascomycota
5	BS1A6	*Alternaria sp.*	Ascomycota
6	BS1A7	*Alternaria sp.*	Ascomycota
7	BS1A10	*Aspergillus sydowii*	Ascomycota
8	BS1B1	*Alternaria sp.*	Ascomycota
9	BS1B2	*Alternaria sp.*	Ascomycota
10	BS1B3	*Alternaria sp.*	Ascomycota
11	BS1B4	*Aspergillus sydowii*	Ascomycota
12	BS1B5	*Fusarium equiseti*	Ascomycota
13	BS1B7	*Alternaria sp.*	Ascomycota
14	BS1C1	*Curvularia geniculata*	Ascomycota
15	BS1C2	*Aureobasidium pullulans*	Ascomycota
16	BS1C3	*Alternaria sp.*	Ascomycota
17	BS1C4	*Mucor sp.*	Zygomycota
18	BS4A1	*Chrysonilia sitophila*	Ascomycota
19	BS4B1	*Alternaria sp.*	Ascomycota
20	BS4C1	*Curvularia geniculata*	Ascomycota
21	BS4C2	*Alternaria sp.*	Ascomycota
22	BS11A1	*Pythium sp.*	Oomycota
23	BS11B1	*Aureobasidium pullulans*	Ascomycota
24	BS11C1	*Mucor micheli*	Zygomycota
25	BS11C2	*Mucor micheli*	Zygomycota
26	BS14A1	*Aspergillus niger*	Ascomycota
27	BS14A2	*Mucor micheli*	Zygomycota
28	BS14A3	*Mucor micheli*	Zygomycota
29	BS14B3	*Mucor sp.*	Zygomycota
30	BS14C2	*Alternaria sp.*	Ascomycota
31	BS14C3	*Chrysosporium xerophilum*	Ascomycota
32	BS22A1	*Alternaria sp.*	Ascomycota
33	BS22A2	*Penicillium sp.*	Ascomycota
34	BS22B1	*Alternaria sp.*	Ascomycota
35	BS22B2	*Alternaria sp.*	Ascomycota
36	BS22B3	*Alternaria sp.*	Ascomycota
37	BS22C1	*Alternaria sp.*	Ascomycota
38	BS22C2	*Aureobasidium pullulans*	Ascomycota
39	BS22C3	*Aureobasidium pullulans*	Ascomycota

Table 3: Percentage Frequency of Occurrence of Different Fungal Genera on the Studied Sorghum Varieties

S/N	Fungal genus	% Frequency
1.	*Aspergillus*	7.23
2.	*Alternaria*	53.01
3.	*Mucor*	12.05
4.	*Penicillium*	2.40
5.	*Aureobasidium*	7.23
6.	*Pythium*	1.21
7.	*Fusarium*	4.81
8.	*Colletotrichum*	1.21
9.	*Chrysosporium*	1.21
10.	*Chrysonilia*	1.21
11.	*Curvularia*	8.43

Table 4: Percentage Frequency of Fungal Infection on the Studied Sorghum Varieties

Sorghum Type	Number of Fungal Colonies	% Frequency of Infection
White	60	40.00[a]
Brown	49	32.60[a]

Means followed by same superscripts within same columns are not significantly different (P≤0.05).

Plate 1. Fungal Colonies Growing on Sorghum Seeds Cultured on Potato Dextrose Agar 3 Days after Inoculation.

DISCUSSION

Fungi belonging to the Division *Ascomycota* and Genus *Alternaria* were the most occurring on the studied stored sorghum varieties. Ascomycetes are currently regarded the largest and most ubiquitous group of fungi on earth. Samson *et al.* (2010) reported that ascomycetous species are often found as storage fungi. The extensive occurrence of ascomycetous fungi on the evaluated grains in comparison to other fungal Divisions could be as a result of their vast adaptability and ability to thrive

on a wider range of nutrient sources compared to members of other Divisions (Anderson, 2018).

Similarly, Leukel and Martin (1943) examined sorghum seeds and found *Alternaria, Aspergillus, Helminthosporium, Penicillium, Rhizopus, Trichoderma,* and *Sphacelotheca.* Christensen and Kaufman (1965) also reported that *Alternaria, Aspergillus, Epicoccum, Fusarium,* and *Penicillium* were associated with grain deterioration under storage conditions. Ellis (1972) also reported the presence of *Alternaria, Curvularia, Helminthosporium,* and *Fusarium* in sorghum grain. Yago *et al.* (2011) reported that members of genus *Alternaria* were the most frequently isolated seed borne mycoflora of sorghum and foxtail millet collected from different growing areas in South Korea. More recently, Wilman *et al.* (2014) reported that *Alternaria spp* were the most frequently isolated seedborne fungi in Poland. The substantial presence of *Alternaria spp*. on the studied sorghum varieties is an indication of the availability of adequate moisture and temperature conditions favourable for spore germination and hyphal development of the fungus in the sampled storage facilities.

The white sorghum variety had a higher fungal load compared to the brown sorghum. The differential and selective preference of white to brown sorghum seeds by seed borne fungi confirmed an earlier report by Thakur *et al*. (2006) of the presence of much lower resistance levels to fungal infections in seeds belonging to the white sorghum variety compared to others.

CONCLUSION

The extensive occurrence of fungi on the stored sorghum suggests the availability of suitable conditions of relative humidity and temperature in storage facilities that are favourable for growth and development of the observed fungi. There is need for emphasis on proper drying and the provision of adequate grain storage facilities for enhanced yield preservation in the study area. Further studies could investigate the role of seed handlers in predisposing sorghum seeds to infections by fungi during storage. There is also need to evaluate the mycotoxin content of stored sorghum seeds in order to ascertain their safety for human and animal consumption in the study area.

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